Conference Report
"Endothelial Cell Heterogeneity and Organ-specificity"[1]

For the second time, a special interest subgroup of approximately 120 scientists met at the Annual Meeting of the American Society of Cell Biology in Denver, Colorado, to discuss endothelial cell (EC) heterogeneity and organ specificity. In his introductory remarks the organizer, P.I. Lelkes (Univ. Wisc. Med. School, Milwaukee, WI) stressed the timeliness of this issue in view of emerging evidence of the role of the microenvironment in modulating the particular phenotype and function of vascular ECs. In addition, characterization of genetic and epigenetic inducers and markers of EC phenotypic diversity will be of relevance for any type of selective, localized pharmacological interventions at the level of the vessel wall.

The successful isolation and to characterization of five morphologically distinct microvascular endothelial cell lines from the corpus luteum suggests the occurrence of a hitherto unknown heterogeneity within the microvasculature, K. Spanel-Borowski Univ. Basel, Switzerland. In addition to morphological differences, these diverseEC types also exhibit individual functional differences, e.g. in terms of cell proliferation in their response to cytokine treatment (A. Fenyves, Univ. Basel). The establishment of unique "markers" will aid in localizing the site of origin of each of these cells. Dr. Spanel-Borowski's intriguing hypothesis of a mosaic-like arrangement of diverse ECs within the same vascular bed seems to be in line with recent studies on the embryonic origin and development of the avian vasculature. In this context, P.I. Lelkes reported on the remarkable degree of organ-specificity and heterogeneity of microvascular ECs in endocrine organs as revealed in vivo by lectin studies. Some of these unique glycosylation patterns are also maintained in low-passage isolated microvascular ECs in vitro. In addition, co-culture studies between isolated EC and parenchymal cells, suggest that organ-specific EC differentiation might require heterotypic intercellular communication, including juxtacrine signals. Thus, EC heterogeneity and organ-specificity might reflect the cumulative expression of post-translational modifications and also the expression of unique genes, which are under the control of organ-specific regulatory elements.

The second group of talks addressed the role of the extracellular matrix (ECM) in regulating EC phenotypic diversity. This interrelationship is bidirectional: Previous work has shown that the composition of a preformed ECM can modulate the EC phenotype. Conversely, ECs can synthesize different sets of ECM proteins, depending on the state of EC "differentiation". M.L.Iruela-Arispe (Univ. Washington, Seattle WA), used the paradigm of EC sprouting and chord formation as a particular model of in vitro angiogenesis to highlight differences in the pattern of ECM protein expression in angiogenic vs. quiescent ECs. The data suggest the occurrence of autoregulatory loops in the phenotypic modulation of ECs. For example, secretion of SPARC promotes, in an autocrine fashion, the angiogenic EC phenotype, by upregulating the secretion of tPA and fibronectin. However, since the process of transformation from a quiescent to an angiogenic phenotype is observed only in some, but not all cultures, crucial questions remain, such as, what triggers certain quiescent ECs to initiate the angiogenic process, what are the single steps that finally lead to the upregulation, and, finally, what switches the angiogenic cell type back to the quiescent one? T.F. Lane (Univ. Washington, Seattle, WA) described several SPARC-derived peptides, generated through proteolytic cleavage (e.g. by plasmin), which in vivo might be involved in initiating and maintaining an angiogenic EC phenotype. A particular peptide sequence $SP_{113-130}$ was mitogenic and rapidly induced in vitro chord formation in cultured ECs. The angiogenic effects, mimicked by an even smaller peptide fragment containing the GHK sequence, were further augmented by addition of Cu^{2+}. These findings could potentially be relevant clinically, since it has been shown that anti-copper therapy might reduce the progression of brain tumors by interfering with tumor angiogenesis.

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[1] The meeting was made possible by generous contributions from GIBCO BRL, Miles Pharmaceutical, SmithKline Beecham Pharmaceuticals, and the Council for Tobacco Research USA.

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Detailed understanding of the functional heterogeneity of ECs e.g., in terms of receptors, signal transduction etc., will be required for precise analysis of the regional regulation of EC physiology and pathophysiology such as in thrombomodulatory or inflammatory responses. T. Moatter (Miles Inc, West Haven CT) described a novel cyclooxygenase (COX II), which is expressed at high levels in large vessel EC, but not in microvessel derived EC. Addition of acidic FGF induced *de novo* synthesis of COX II in the microvessel ECs via a PKC dependent pathway. By contrast, in large vessel ECs exposure to aFGF resulted in a decrease in cyclooxygenase, suggesting the possibility of differential signal transduction mechanisms in various ECs. M. E. Gerritsen (Miles, New Haven, CT) illustrated several kinds of divergent functional responses of micro- and macro-vessel ECs upon stimulation with cytokines, such as Il-1, and TNF-α. For example, in large vessel ECs cytokine treatment induces urokinase activation and increases the expression of cell adhesion molecules, such as ICAM-1. However, in synovium- or lung-derived microvessel EC, which have high constitutive levels of urokinase, the same cytokines did not alter uPA levels and ICAM-1 levels were only modestly affected. Cell type specific, divergent responses to concomitant exposure to different cytokines might also reflect the prevalence of different signal transduction pathways.

It is well known that inflammatory stimuli modulate local proteolysis, e.g., by altering the balance of endothelial cell-derived thrombomodulatory proteins, such as tissue plasminogen activator and its major inhibitor, plasminogen activator inhibitor type 1 (PAI-1). D.L. Amrani (U. Wisconsin, Milwaukee, WI) described the unique role of IL-6 in regulating PAI-1 gene expression in microvessel-derived ECs. Exposure to lipopolysaccharide (LPS) caused the production of both PAI-1 and IL-6. Indeed, stimulation of these cells with IL-6 alone in the presence and absence of neutralizing α-IL-6 antibodies suggests that LPS-stimulated PAI-1 production is primarily due to an IL-6 induced autocrine response. By contrast, in large vessel EC, PAI-1 levels are up-regulated by IL-1 or TNF-α, but not by IL-6. Differential regulation of the thrombomodulatory responses of EC from distinct anatomical sites suggests a particularly tight mode of controlling fibrinolysis in the microvasculature.

Unlike most other cells, ECs reside in a dynamic environment. Local hemodynamic parameters, in particular flow -induced shear stress, play a major role in the differential modulation of EC morphology and function in vivo and in vitro. Another component of the dynamic environement, cyclic strain, differentially modulates EC functions, such as rate of proliferation, protein synthesis, or the production of vasomodulators, depending on the origin of the ECs I. Mills (Yale Univ. School Medicine, New Haven, CT), . The effects of cyclic stress are mediated through well known pathways of intracellular signal transduction, (activation of protein kinases, and phosphoinositide cascade), which seem to be common for all EC types studied so far. However, stretch-activated cAMP production seems to be restricted to arterial ECs only and is absent in venous ECs. Using static controls and cyclic stretching, P.I. Lelkes demonstrated heterogeneous cAMP signalling in various ECs: Depending on their origin within the vascular bed, EC differ in their basal cAMP levels, their responses to α-adrenergic agonists and other activation of adenylate cyclase, their responsiveness to cyclic strain and also in terms of the synergism between chemical and mechanical activation. These findings might be explained by assuming that phenotypically diverse EC might express some of the isoforms of the various components of the cAMP signalling machinery. On the other hand these findings also support the recurring notion that EC heterogeneity might comprise differential availability and/or efficacy of certain common intracellular signal transduction pathways. Furthermore, differential susceptibility to mechanical stimulation hints at the possibility that EC heterogeneity resides in the mechanism by which the cells perceive exogenous (mechanical) signals: Is there a set of specialized (heterogeneous) mechanoreceptors which in turn selectively activate mechano-responsive elements? Next year's meeting might bring us closer to an answer.

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